THE GOLGI APPARATUS OF AMOEBA PROTEUS PALLAS

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Introduction

The study of the Golgi apparatus and its identification in the Protozoa has been rendered almost impossible because the highly specialized Golgi techniques are not very specific, and they are very capricious even in the hands of our best technicians. Bowen (1928) has pointed out the fact that isolated gland cells often fail to respond to impregnation methods, and he suggests that similar trouble may be expected in protozoan techniques. Also the identification of the Golgi apparatus is impeded because just what may or may not be Golg. material has not been agreed upon by many of the investigatorsi Hirschler (1914) found spheres, crescents, and rings in *Monocystis agilis* and *Gregarina polymorpha*. King and Gatenby (1923) described similar crescents, and bead-like structures in *Adelia*. They believe these structures to be comparable to the dictyosomes of metazoan cells. Nassonov (1924, 1925) believes that the contractile vacuole is the homologue of the Golgi apparatus of metazoan cells. He found that the contractile vacuoles of *Paramecium*, *Lionotus*, *Chilodon*, and *Dogielella* stained by the Kolachev method of procedure. Duboscq and Grassé (1924–1927) believe that in *Holomastigotes*, *Pyrsonympha* and other related flagellates the parabasal bodies are the homologues of the Golgi apparatus. Grassé (1926) stated that since the euglenoid flagellates have no parabasal bodies, the stigma or eye-spot is a homologue of the Golgi apparatus. Causey (1925) finds a network in *Endamœba gingivalis;* this structure is similar to the Golgi network in metazoan tissues. Hirschler (1927) found rings, spheres, and crescents in *Endamœba blattæ* and he believes that these are the Golgi material. Joyet-Lavergne (1926) used neutral red as a vital stain on gregarines and found that these crescents and rings stained; therefore, he believes the structures to be Golgi elements. Hall (1930) finds in *Chilomonas paramecium* small granules and large vacuoles, and he believes that these bodies are comparable to the Golgi apparatus.

There is a great divergence of opinion as to just what may be or may not be Golgi material in the Protozoa. The structure and function of the Golgi apparatus are subjects of controversy. It is the aim

of the writer to describe and discuss the Golgi apparatus of *Amœba proteus* Pallas.

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Material and Technique

Amœba proteus was cultured on a cracked wheat infusion which was inoculated with *Chilomonas paramecium* and *Zoöchlorella*.

For fixation the centrifuge method of procedure was used. The Kolachev method as modified by Nassonov (1925) and Bowen's modification of the Mann-Kopsch procedure were used to good advantage. The Mann-Kopsch method gave the best results. Also Bowen's acid fuchsin-thionin-aurantia was slightly modified and used after a fixation in Champy's solution.

(1) Fix in Champy's solution 12 hours.
(2) Wash in distilled water.
(3) Pass the slides through graded alcohols to 70 per cent.
(4) Stain 10 minutes in acid fuchsin.
(5) Stain 15 seconds in very dilute thionin.
(6) Stain 5 seconds in dilute aurantia.

Dehydrate and mount in balsam.

Result: Golgi bodies are light blue with dark blue rims, the cytoplasm is pink, the nucleus is orange or vermillion, and the contractile vacuole and the granules around it are red.

The Golgi Apparatus of Amoeba

The Mann-Kopsch method of procedure brings out two types of granules in *Amœba proteus* which reduce osmic acid. One of these is a black granule and the other is a spherule with a black rim (Fig. 2, Plate). The black granules are similar to those described by Hall (1930) as occurring in *Chilomonas paramecium*. When the spherules are viewed at a central focus, they appear as rings, or crescents (Figs. 1–2, Plate). These bodies have clear centers. Both types are distributed at random throughout the endoplasm of *Amœba*, and often they have a tendency to flow out into the pseudopodia (Fig. 2, Plate). In no cases have they been observed to be associated with the contractile vacuole. There are small granules which group around the contractile vacuole. These do not take an osmic stain, but they are easily demonstrated by acid fuchsin (Fig. I, Plate).

The contractile vacuole of *Amœba proteus* does not take an osmic

stain; however, *Paramecium caudatum* occurs in the same slides and its contractile vacuoles are blackened by osmic acid as described by Nassonov (1925). Small vacuoles of variable sizes have been observed to be attached to the spheres with dark rims (Figs. 1–2, Plate). These are also noticed in the endoplasm; they are numerous around the contractile vacuole (Figs. 1–2, Plate). This causes the writer to believe that these small vacuoles originate from small granules which move about in the endoplasm and group around the contractile vacuole. They evidently flow together to form the new vacuole after the systole. The formation of the contractile vacuole is different therefore, from *Paramecium caudatum*, where the contractile vacuoles with their feeding canals are more or less permanent structures. The black granules are of variable sizes, and it is possible that these grow in size to form the spheres with black rims. The reaction of the acid fuchsin-thionin-aurantia and osmic acid procedures indicate that both types of bodies are Golgi material (Figs. 1–2, Plate). Also these bodies are similar to those described by Hirschler (1914), King and Gatenby (1923), Joyet-Lavergne (1926) and Hall (1930).

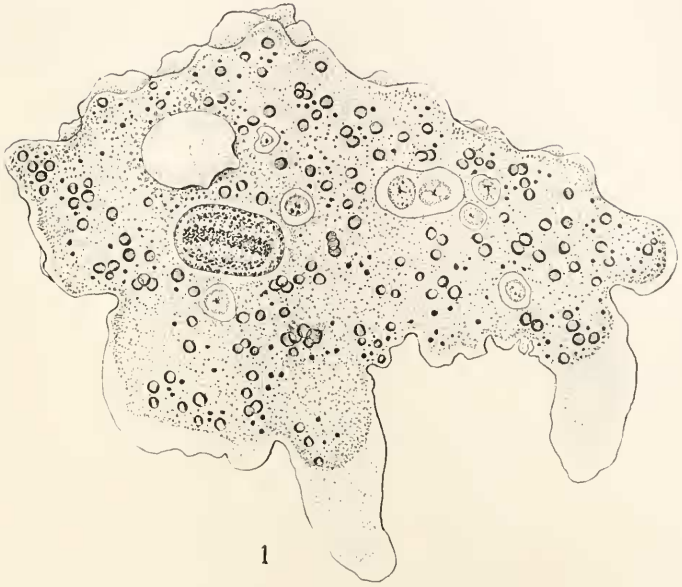
Bowen's modification of the acid fuchsin-thionin-aurantia method of procedure stains the Golgi apparatus of *Amœba proteus* blue. The spheres have dark blue rims (Fig. 1, Plate), whereas their centers are light blue. The Mann-Kopsch procedure stains them black; the large ones have light centers with black rims (Fig. 2, Plate).

Discussion

Hirschler (1914) described the Golgi apparatus (*i.e.*, spherules) of *Monocystis ascidæ* as rings and crescents. These have dark rims with centers. King and Gatenby (1923) described similar bodies as occurring in *Adelia*. They declared that these crescents and bead-like structures were comparable to the dictyosomes of metazoan cells. They also stated that these dictyosomes divide like those of the metazoan cells.

Hirschler (1914) found that the Golgi material of the Protozoa was made up of two substances, one of which takes a light stain, the other a dark one. Such a staining reaction occurs in the Golgi apparatus of *Amœba proteus*. One portion is chromophilic and the other chromophobic. This probably accounts for the fact that the spherules have dark rims. However, the writer does not find that the chromophobic portion has an affinity for acid fuchsin.

It is probably of interest to remark here that Vonwiller (1913) finds that neutral red used as a vital stain on *Amœba* colors the crystal-vacuoles (vacuoles which contain the characteristic crystals of *Amœba*)



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Explanation of Plate

Both figures were drawn with the aid of an Abbé camera lucida. The chondriosomes were omitted in the drawings in order to avoid confusion.

FIG. 1. *Amœba proteus* fixed in Champy's solution and stained with acid fuchsin-thionin-aurantia. The Golgi bodies occur in clumps. The large globules have clear centers and dark rims. This gives the appearance of rings and crescent-shaped structures. The small globules are dark blue.

FIG. 2. *Amœba proteus* fixed and stained by Bowen's modification of Mann-Kopsch procedure. The Golgi bodies have a tendency to flow out into pseudopodia.

orange, whereas the smaller granules stain red. Joyet-Lavergne (1926) finds that vital staining with neutral red brings out the Golgi apparatus in the gregarines. These bodies occur as rings, crescents and spherules with dark red rims. Hall (1929) finds that neutral red used as a vital stain demonstrates the Golgi apparatus in various Protozoa. Hall (1930) finds similar globular bodies in *Trichamœba*, which react to neutral red and osmic acid. Occasionally crescent-shaped structures are noticed. Hall (1930*b*) shows that these globules are stained selectively with neutral red and are the same bodies which are blackened by osmic acid and silver impregnation. He used a mixture of Janus green and neutral red to distinguish these globules from the mitochondria of *Trichamœba*. The writer finds similar globules and crescent-shaped structures in *Amœba proteus* (Figs. 1–2, Plate). These bodies are smaller than the "crystal vacuoles," as described by Vonwiller (1913), but they are probably the same globules which stain bright red. These bodies have a tendency to flow out into the pseudopodia as described by Vonwiller. Therefore these globular bodies and crescent-shaped structures are probably the Golgi apparatus of *Amœba proteus* (Figs. 1–2, Plate).

Bowen (1923–1929) has suggested that the Golgi apparatus has a secretory function. He finds that the Golgi apparatus of glandular tissue hypertrophies at the beginning of the secretory cycle; when it hypertrophies, spherical and globular bodies are formed. These globular bodies have clear centers and dark rims when impregnated with osmic acid and are similar in many respects to those found in the Protozoa. A true Golgi network is not found in *Amœba proteus*, and these crescents and the globules are similar to the globules described by Bowen (1926). The writer is led to believe that the Protozoa are cells where secretion is a constant process; therefore, the Golgi apparatus would naturally be expected to be hypertrophied and occur as globules.

It has been suggested by Bowen (1926) that the relation between the Golgi apparatus and the secretory granules is homologous to that existing between the Golgi apparatus and the developing acrosome of vertebrate sperm, and this latter phenomenon can be used as a basis for interpretating the phenomena in the gland cell. The writer believes that a similar relationship exists between the crescent-shaped Golgi bodies and the minute vacuoles which occur throughout the endoplasm of *Amœba proteus*. These minute vacuoles are often attached to these crescent-shaped bodies (Fig. 2, Plate). They also occur throughout the endoplasm, and similar vacuoles occur in large numbers around the contractile vacuole. These vacuoles break into the contractile vacuole when the systole occurs. Day (1927) finds that the

contractile vacuole of *Amœba proteus* arises from the fusion of small vacuoles which probably owe their origin to the fusion and coalescence of ultra-microscopic droplets of soluble katabolic waste which may include the water of osmosis. The minute vacuoles described above seem to be similar in all respects to those described by Day (1927). The vacuole of *Amœba* is not a permanent structure and is formed by the fusion of minute vacuoles; this differs from the contractile vacuoles of *Paramecium caudatum*, where two permanent contractile vacuoles occur and pulsate successively. The contractile vacuoles of *Paramecium caudatum* have long "feeding" canals, and these structures are blackened by osmic acid (Nassonov, 1925).

Paramecium occurs in my slides along with *Amœba proteus*, and the contractile vacuoles of *Paramecium* are blackened by osmic acid; whereas, the contractile vacuole of *Amœba proteus* is not stained. This may be due to the difference in the formation of these vacuoles in the two Protozoa.

General Summary

1. The Golgi apparatus of *Amœba proteus* is the characteristic protozoan type of globules and spherules with clear centers and dark rims. These spherules, from a central focus, appear to be crescent-shaped structures. Small black granules appear without black centers.

2. These Golgi bodies are readily blackened by osmic acid and stained with thionin.

3. At the beginning of the secretory cycle of metazoan gland cells, the Golgi apparatus hypertrophies and globules are formed; the globules of *Amœba proteus* are believed to be homologous to these structures.

4. Since the secretory cycle is a continuous process in *Amœba*, it is suggested that this is the reason for the absence of a Golgi network.

5. It is suggested that the minute vacuoles which occur in the endoplasm of *Amœba* are associated with the crescent-like Golgi bodies, a relationship which is similar to that existing between the metazoan Golgi apparatus and the secretory cycle.

6. The contractile vacuole of *Amœba* is formed by a union of these minute vacuoles. It is possible that this is the reason why the contractile vacuole of *Amœba* is not blackened by osmic acid like the contractile vacuoles of *Paramecium caudatum*.

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